concentrations of pituitary hormones from all three lobes of the pituitary, in the long portal vessels. Functional significance of these observations remains to be proved; there is as yet no evidence that blood flows retrograde from pituitary into brain in a functional mechanism able to deliver pituitary peptides. On the contrary, we have evidence that profound variations in the pituitary secretion of β-endorphin are not reflected in concomitant variations of brain levels of β-endorphin (*14*). Thus, with all this conflicting evidence, we have to search for one or several peripheral targets for the β-endorphin secreted in response to stress in the dynamic fashion and large amounts that we have demonstrated here.

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Antiallatotropins: Inhibition of Corpus Allatum Development

Abstract. *Treatment of newly eclosed adult milkweed bug* (Oncopeltus fasciatus) *females with precocene 2 prevents secretion of juvenile hormone by inhibition of postimaginal development of the corpus allatum. Ovarian development which is dependent upon juvenile hormone is prevented or reversed, depending upon the timing of precocene treatment. Juvenile hormone secretion is shown to be related to the development of the corpus allatum.*

The juvenile hormones (JH) of insects regulate insect metamorphosis and reproduction (*1*). Treatment of several heteropterous insect species with certain simple chromenes, called precocenes (Fig. 1) induces precocious metamorphosis of the immature stages and prevents ovarian development in the adult stage. Since these effects are fully reversible by treatment with JH, the precocenes are clearly blocking the normal physiology of JH during synthesis, release, transport, or at its site of action. In view of these actions the precocenes have initially been called anti-juvenile hormones (*2*, *3*).

Although the precocenes produce the full range of anti-juvenile and anti-gonadotropic actions against many heteropterous insects, other insect groups, especially Lepidoptera, Coleoptera, and Diptera, may be sterilized in the adult stage but do not undergo precocious metamorphosis. An understanding of the mode of action of the precocenes is crucial to the further development of methods that interfere with normal endocrine function and may thus be used for insect control. Since the actions of the precocenes can be reversed by treatment with JH, it seemed possible that precocenes produce their anti-juvenile hormonal actions through interference with the secretion of JH by the corpora allata.

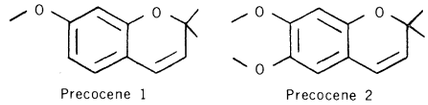


Fig. 1. Anti-juvenile hormones: precocene 1 and 2.

Fluctuations in the volume of the corpora allata have been correlated with cyclic ovarian growth and regression in several insects (*4*). In the milkweed bug, Johansson (*5*) has shown that the corpora allata are very small at eclosion and rapidly enlarge to a maximum volume prior to ovarian development, but the allatal volume does not decrease significantly even during senescence. Johansson has also reported that starved females fail to develop their ovaries, and allatal volume remains subnormal. Since these data taken together suggest that the corpora allata must undergo a postimaginal period of development prior to JH secretion, we investigated the effect of precocene 2 on allatal volume and oocyte development.

We found that precocene treatment of newly eclosed female milkweed bugs prevented allatal development and that the ovaries of treated insects fail to grow, indicating that the undeveloped allata fail to secrete JH. Furthermore, precocene treatment of mature females stops ovarian development and induces regression of the corpora allata.

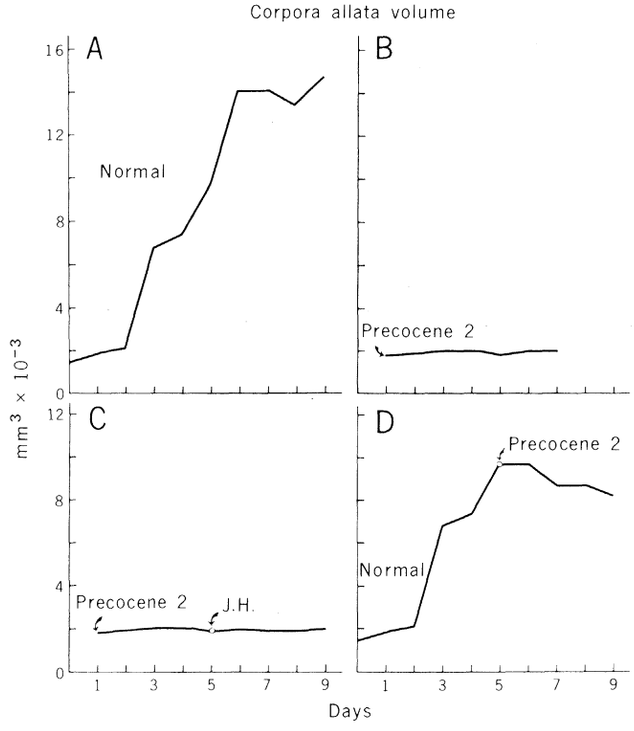
Newly eclosed milkweed bug (*Oncopeltus fasciatus*) adult females were collected from a stock culture. Groups of ten insects were treated within 24 hours after eclosion or at 120 hours by contact with 8 μg/cm² of precocene 2 in a petri dish for 48 hours as reported previously (*2*), and then transferred to untreated dishes for the remainder of the trials. Insects were maintained on milkweed seeds and water during and after treatment. Juvenile hormone III (10 μg in 1 μl of acetone) was applied topically to the abdomens of some insects. Control insects received acetone only. All experiments were performed in duplicate.

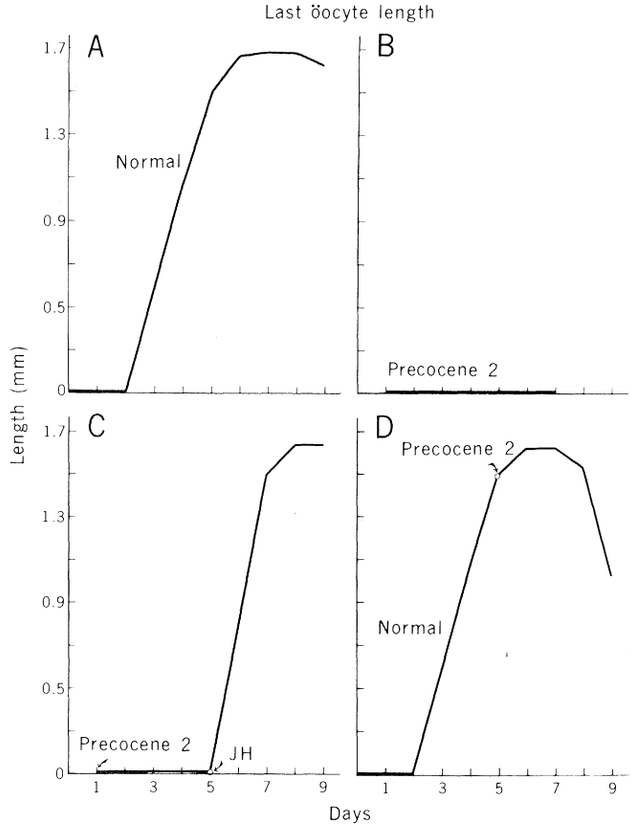
The corpora allata were dissected under Ringer solution and measured with an ocular micrometer, and their volume was calculated as the volume of a sphere. Ovarian development was determined by measurement of the length of the last oocyte of the ovaries with the ocular micrometer.

The volume of the fused corpora allata increased rapidly (Fig. 2A) during postimaginal development, reaching maturity at 6 days. Under our rearing conditions (26°C, 76 percent relative humidity, 16 hours of light, 8 hours of darkness) oviposition occurred on day 6. If insects were treated with precocene on day 1 the corpora allata did not develop (Fig. 2B). When juvenile hormone was applied to precocene-treated insects at 120 hours, there was no effect on the volume of the corpora allata (Fig. 2C). However, as shown in Fig. 2D, when normally devel-

oping females were treated with precocene at 120 hours allatal development ceased and the corpora allata underwent subsequent regression.

Oocyte development paralleled the growth of the corpora allata as evidenced by their rapid growth to a maximum at 6 days (Fig. 3A). Precocene treatment within 24 hours of eclosion completely eliminated oocyte growth (Fig. 3B), whereas in C rapid oocyte growth in precocene sterilized insects followed topical administration of JH at 120 hours (Fig. 3C). When normal insects were treated with precocene at 120 hours (Fig. 3D) oocyte growth continued for 1 day and then stopped and began to regress. Several females in this group were able to oviposit successfully on day 6 or day 7; however, oviposition then ceased and dissection revealed that their ovaries had completely regressed. Since ovarian growth is dependent upon JH secretion, precocene inhibition of allatal development must stop the flow of JH. The results of these studies are summarized in Fig. 4.





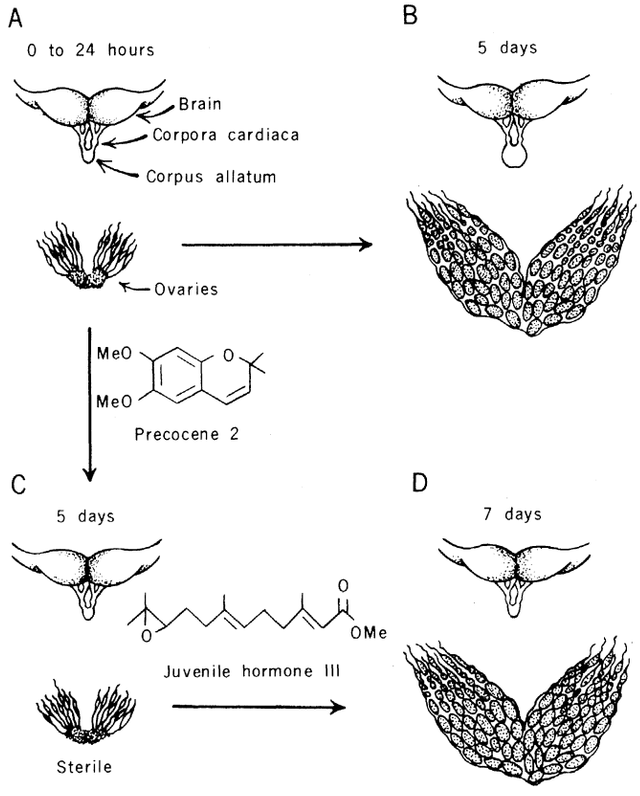


Fig. 2 (top left). Precocene inhibition of corpus allatum development in adult female milkweed bugs. (A) The volume of the corpus allatum increases rapidly to a maximum at 9 days after eclosion. (B) Allatal development is prevented by precocene treatment on the day of eclosion. (C) Treatment of precocene-sterilized females with JH III on day 5 does not affect allatal development. (D) Treatment of normal females on day 5 after eclosion with precocene stops allatal growth and causes regression. Fig. 3 (top right). Precocene inhibition of oocyte development in adult female milkweed bugs. (A) Oocyte development begins between day 2 and day 3 after eclosion and reaches maximum on day 6. Oviposition normally begins on day 6. (B) Oocyte growth is completely inhibited after treatment with precocene on day 1. (C) Treatment of precocene-sterilized females on day 5 with JH III induces rapid oocyte development and oviposition occurs on day 7. (D) Treatment of normal females with precocene on day 5 causes regression of the oocytes, although a small number of viable eggs may be laid on day 6. Fig. 4 (bottom left). Summary of precocene action on the development of the corpus allatum and oocytes of adult female milkweed bugs. (A to B) Within 5 days after eclosion the corpus allatum increases dramatically in size. Allatal development coincides with JH secretion, since the ovaries also reach full development within this period. (A to C) Treatment of newly eclosed females with precocene prevents allatal development. The undeveloped corpus allatum does not produce JH, as evidenced by the lack of oocyte growth. (C to D) Treatment of precocene-sterilized females with JH III does not overcome the allatal inhibition, but the ovaries are induced to develop fully demonstrating that the ovaries are poised target organs capable of responding to JH.

Precocene unequivocally inhibits or stops allatal development at any stage of application since precocene treatment can both prevent JH secretion when applied to newly eclosed females, or it can stop allatal production of JH if applied to sexually mature females. The fact that functional corpora allata can be stopped from secreting JH explains the precocious metamorphosis of precocene-treated nymphal stages.

These studies confirm that the size of the corpora allata is linked to JH secretion and that the ovaries are dependent upon JH for development, and show that the ultimate action of precocene prevents allatal growth and JH secretion. Since the ovaries of precocene-sterilized insects remain fully poised to respond to exogenous JH, it appears that precocene has no direct action upon the ovary.

Since precocene stops JH secretion through inhibition of allatal development, we suspect that precocene interferes with humoral or nervous regulation of the corpora allata. For the precocenes, therefore, we suggest the term antiallatotropin rather than anti-juvenile hormone since the latter term is more general and could encompass other modes of hormone antagonism including interference with JH biosynthesis, transport, or action at a receptor site.

Although these studies show that precocene prevents JH secretion by inhibiting allatal development, we have not demonstrated whether precocene acts directly upon the corpora allata or whether its action is mediated through some other organ that is its actual target. Numerous investigations have established the brain as the prime regulator of the corpora allata (*6*), and therefore the brain may be the actual target of precocene. Should this be the case, it may be possible to utilize precocene in the investigation of brain-allatal interactions and the development of new insect-growth regulators.

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Expression of Murine Sarcoma Virus Genes in Uninfected Rat Cells Subjected to Anaerobic Stress

Abstract. *Exposure of uninfected rat cells in tissue culture to anaerobic culture conditions induces transcription of RNA corresponding to the two principal constituents of rat-derived type-C sarcoma virus genomes: (i) those specific rat cell sequences present in the Kirsten and Harvey murine sarcoma virus genomes, and (ii) an endogenous type-C rat leukemia virus.*

Increasing evidence in both murine and avian systems indicates that the genomes of the highly oncogenic type-C sarcoma viruses are composed of normal cellular genetic information, recombined in an abnormal form. A major portion of the sarcoma virus genomes represents type-C leukemia viruses, which in turn have been shown to exist in a latent but inducible form within normal cell DNA (*1*). Such leukemia viruses are only weakly oncogenic in that they have long latent periods for tumor induction in vivo and generally fail to transform cells in culture (*2*). Specific additional cellular RNA sequences recombined with leukemia virus genes have been identified in sarcoma virus genomes (*3*, *4*). Since type-C sarcoma viruses readily transform cultured cells, quickly induce tumors in vivo, and carry at least one defined transforming gene (*5*), characterization of sarcoma virus gene function offers a reasonable hope of explaining how viruses can directly induce cancer. We report here studies aimed at elucidating sarcoma virus gene function, particularly by identifying those variables which influence the expression by normal cells of sarcoma virus genome components.

The Kirsten and Harvey murine sarcoma viruses represent recombinants between the type-C mouse leukemia viruses with which each was first isolated (Kirsten murine leukemia virus and Moloney murine leukemia virus) and the same set of specific rat cellular genetic information (*3*, *6*). With techniques described elsewhere (*3*), we have used a [^{3}H]thymidine-labeled reverse transcrip-

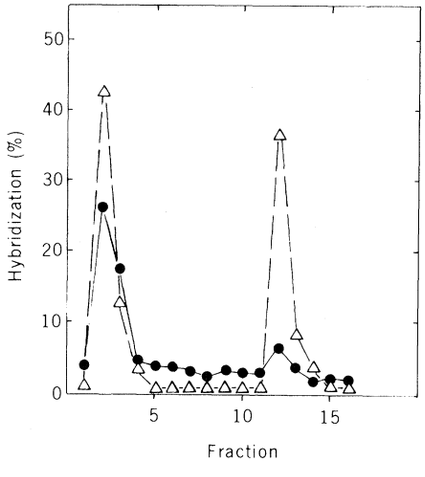

Fig. 1. Oligo(T) cellulose column chromatography of Fischer rat cell RNA. RNA (0.8 mg) was loaded on a 2.2-ml column containing 0.7 g of oligo(T) cellulose (Collaborative Research, Waltham, Mass.), according to the procedure of Scolnick *et al.* (*19*). The nonadsorbed material was removed by elution with 0.5*M* KCl, 0.01*M* tris, *p*H 7.5, in ten 2-ml fractions. The bound material was eluted in 0.01*M* tris, *p*H 7.5, in six 2-ml fractions. After monitoring the absorption of each fraction at 260 nm (98.6 percent of the input RNA eluted in the void, 1.4 percent was adsorbed on the column), 300 μg of yeast transfer RNA and two volumes of ethanol were added to each fraction. The precipitated RNA was resuspended in 100 μl of 0.1*M* NaCl, 0.01*M* tris, *p*H 7.5, and 0.001*M* EDTA. ^{3}H-Labeled DNA products of the Kirsten murine sarcoma virus (KiMSV) and the Wistar-Furth (WFU) rat leukemia virus were prepared and used as molecular hybridization probes as described elsewhere (*3*). Hybridization to WFU-RaLV [^{3}H]DNA is expressed directly, while that to KiMSV (KiMuLV) [^{3}H]DNA is normalized to its rat-specific fraction (40 percent). Hybridization was carried out in a 50-μl reaction mixture containing [^{3}H]DNA (2×10^3 count/min; 0.3 ng) and 20-μl resuspended column fractions in a buffer composed of 38 percent formamide, 600 m*M* NaCl, 50 m*M* 2-{[tris(hydroxymethyl)]-methylamino}ethanesulfonic acid (TES), *p*H 7.5, and 1 m*M* EDTA. After incubation for 7 days at 43°C, hybridization was monitored with nuclease *S*1. Increased quantities of oligo(T) cellulose column fractions 2 and 12 were able to hybridize over 90 percent of each [^{3}H]DNA. Plain cellulose [without coupled oligo(T)] gave no retention of either RNA species. Symbols: ●, hybridization to WFU-RaLV [^{3}H]DNA; △, hybridization to the rat-specific fraction of the [^{3}H]DNA product of KiMSV (KiMuLV).